1. INTRODUCTION

1.1 Background-Clinical

Prostate cancer is the second leading cause of cancer death among males in the United States. There will be an estimated 180,000 new diagnoses of prostate cancer made in the United States in 1999 (1). Treatments for organ-confined prostate cancer include radical prostatectomy and radiation. Once the cancer extends outside the prostate, the only systemic treatment is the manipulation of androgens, a treatment that will result in growth inhibition of cells that are androgen-dependent, but has no effect on the population of malignant cells that are androgen-independent. These androgen-independent prostate tumor cells continue to grow and metastasize.

Anti-tumor therapies for cancer, including prostate cancer, have been limited, prompting many scientists to investigate the use of immunotherapies and gene therapies. Most of the immunotherapy studies for prostate cancer have concentrated on active non-specific therapy and adoptive or passive therapy, with only recent attention paid to the induction of antigen-specific immune responses. It is our contention that active immunization against antigens associated with prostate cancer will be more effective than active non-specific or adoptive/passive immunotherapy. Therefore, we have been pursuing a vaccination strategy based on an adenovirus that carry the gene for prostate specific antigen (PSA).

Viral vectors have been used successfully in both gene transfer and vaccine therapy studies (2). Replication competent and replication deficient adenoviruses expressing foreign proteins have been used to elicit immune responses to a variety of tumor antigens (3). We have been able to demonstrate that immunizations with adenovirus, carrying the human PSA gene, can induce vigorous anti-PSA T-cell responses and cause the destruction of PSA-secreting tumors in a pre-clinical mouse model of prostate cancer.

We propose in this protocol to conduct a Phase I clinical trial of adenovirus/PSA (Ad/PSA) vaccine in men with prostate cancer. This will be a dose escalation, administered either subcutaneously (sc) in an aqueous solution or in a collagen matrix (Gelfoam®).

1.2 Adenovirus Vector

Recombinant adenoviral vectors transduce a wide range of dividing and nondividing cells types, making this gene delivery system valuable as a tool for studying diseases, for vaccine therapy, and for potential clinical use (4). Recombinant adenovirus can be prepared and purified in high titers. In addition, wild-type adenovirus infections are extremely common in the general population, giving adenovirus a well-documented safety record (5). Moreover, adenovirus are structurally stable and no side effects have been reported following the vaccination of US military recruits with wild types, demonstrating their safety for human use (6). Adenoviral vectors for gene therapy and vaccine therapy are adenovirus which have been genetically modified to allow insertion of foreign genes and to render the virus replication-defective. Current vectors have a deletion in the E1 region or in both the E1 and E2 regions. Adenoviral gene transfer has been used in a variety of experimental conditions that include transfers to the liver (7), lung (8), central nervous system (9, 10), and to cancer cells (11).

There is evidence that the introduction of foreign transgenes by adenovirus induces a CTL response to the transgene product that is ultimately responsible for the elimination of the virus (4, 12). While this is disadvantageous in situations where gene

therapy is being used to insert functional genes into host cells, it is advantageous in the use of viruses carrying foreign genes as immunogens. In the vaccine therapy of cancer, active immunization against a murine colon cancer antigen and melanoma antigens have been induced by adenoviral vaccines (13, 14).

1.3 Gelfoam® Matrix

Gelfoam (Pharmacia & Upjohn Company, Kalamazoo, MI) is a medical device intended for application to bleeding surfaces as a hemostatic agent. It is a water-insoluble, off-white, non-elastic, porous, pliable product prepared from purified pork skin. The Gelfoam gelatin preparation is available either as a cross-linked sponge or as non-cross linked beads. It is able to absorb and hold within its interstices approximately 45 times its weight of blood and other fluids (15). The absorptive capacity of Gelfoam is a function of its physical size, increasing with increasing gelatin volume (16).

The mechanism of action of surface-mediated hemostatic devices is supportive and mechanical (16). Surface-acting devices, when applied directly to bleeding surfaces, arrest bleeding by the formation of an artificial clot and by producing a mechanical matrix that facilitates clotting (17). Jenkins et al have theorized that the clotting effect of Gelfoam may be due to release of thromboplastin from platelets, occurring when platelets entering the Gelfoam become damaged by contact with its myriad of interstices (18). Thromboplastin interacts with prothrombin and calcium to produce thrombin, and this sequence of events initiates the clotting reaction. The authors suggest that the physiologic formation of thrombin in Gelfoam is sufficient to produce formation of a clot, by its action on the fibrinogen in blood (18). The spongy physical properties of Gelfoam hasten clot formation and provide structural support for the forming Clot (17, 19).

Gelfoam has been used experimentally for the delivery of soluble proteins and drugs, including insulin, antibiotics, and growth factors (20-22). Gelfoam was used for sustained release of insulin in an ocular implant device (20). Delivery of insulin in solution had no effect on blood glucose levels. In contrast, the use of Gelfoam as a sustained release delivery agent provided measurable insulin activity for up to 10 hours after implantation. Glucose levels in the blood stabilized at 60% of the original value, whereas administration of insulin in eye drops had no effect.

MacDonald and Mathews (23) studied Gelfoam implants in canine kidneys and reported that it assisted in healing, with no marked inflammatory or foreign-body reactions. Jenkins and Janda (24) studied the use of Gelfoam in canine liver resections and noted that Gelfoam appeared to offer a protective cover and provide structural support for the reparative process. Correll et al (25) studied the histology of Gelfoam when implanted in rat muscle and reported no significant tissue reaction.

Gelfoam has been used in prostate. Hanisch and associates (26) used Gelfoam as a hemostatic agent in dog prostate. In these studies no gross histological evidence of tissue damage or calcification was induced. In addition, these investigators demonstrated that placement of Gelfoam into the lumen of the bladder resulted in liquefaction of the Gelfoam without any evidence of calculogenesis. Finally,Bischoff and Goerttler (27) used Gelfoam in human prostate therapeutic embolization with success.

Our laboratory, in collaboration with Dr. Timothy Ratliff, has demonstrated that administration of the Ad/PSA vaccine in Gelfoam induces a stronger anti-PSA immune

response. In our pre-clinical studies, immunization with the vaccine in an aqueous suspension induces strong immunity with 10^9 and 10^8 pfu with weaker immunity induced with 10^7 pfu. Use of Gelfoam permits the induction of strong responses at the lower dose of 10^7 pfu. This information can be found in the separate report on our pre-clinical studies.